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**Legacy of a chemical factory site: Contaminated groundwater impacts
stream macroinvertebrates**

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14 **ABSTRACT**

15 Legislative and managing entities of EU member states face a comprehensive task since the
16 chemical and ecological impacts of contaminated sites on surface waters need to be assessed. The
17 ecological assessment is further complicated by the low availability, or in some cases, absence of
18 ecotoxicity data for many of the compounds occurring at contaminated sites. We studied the
19 potential impact of a contaminated site, characterised by chlorinated solvents, sulfonamides and
20 barbiturates, on benthic macroinvertebrates in a receiving stream. Most of these compounds are
21 characterised by low or unknown ecotoxicity, but they are continuously discharged into the stream
22 via a long-lasting source generating a long-term chronic exposure of the stream biota. Our results
23 show that taxonomical density and diversity of especially sediment dwelling taxa were reduced by
24 > 50% at the sampling sites situated in the primary inflow zone of the contaminated groundwater.
25 Moreover, macroinvertebrate communities at these sampling sites could be distinguished from
26 upstream control sites and sites situated along a downstream dilution gradient using multi-
27 dimensional scaling. Importantly, macroinvertebrate indices currently used did not identify this
28 impairment, underpinning an urgent need for developing suitable tools for the assessment of
29 ecological effects of contaminated sites in streams.

30

31 **Key words:** Groundwater contaminants, contaminated sites, macroinvertebrates, morphological
32 deformities, Chironomidae, Chlorinated solvents

33 INTRODUCTION

34 Historical depositions of environmental contaminants on e.g. industrial sites and landfills may be
35 transported to surface water via groundwater. The transport of xenobiotic organic compounds from
36 these types of contaminated sites to surface waters will differ in space and time depending on the
37 amount of deposited compounds and the connectivity of the sites with groundwater (Conant et al.
38 2004; Milosevic et al. 2012; Westbrook et al. 2005). According to the EU Water Framework
39 Directive (Directive 2008/105/EC), member states are obliged to assess the risk of all contaminated
40 sites that may impact chemical and ecological quality of streams and lakes via groundwater
41 transport. Ideally, the ecological impairment in streams caused by contaminated sites should be
42 disentangled from the effects of other stressors, such as diffuse source pollution from agricultural
43 and urban settings, in order to evaluate their importance (McKnight et al. 2012; Rasmussen et al.
44 2013; Roy and Bickerton 2012; Tesoriero et al. 2013).

46 Contaminated sites near surface water bodies may harbour a substantial array of compounds,
47 including e.g. chlorinated solvents, gasoline constituents, pharmaceutical compounds, metals and
48 metalloids, and pesticides (Chapman et al. 2007; Dickman and Rygiel 1998; McKnight et al. 2010;
49 Milosevic et al. 2012; Westbrook et al. 2005). Only a fraction of these contaminants are routinely
50 monitored in streams (especially pesticides and metals) while other compounds may be overlooked
51 because they fail to meet the central criteria for ecological concern (toxicity, persistence and
52 bioaccumulation). Importantly, contaminants with low predicted ecotoxicity to aquatic biota may
53 still be harmful when the contaminants are continuously discharged into surface waters via long-
54 lived sources (e.g. on the order of tens to hundreds of years) resulting in year-long chronic exposure
55 scenarios (Conant et al. 2004; Daughton 2005; Weatherill et al. 2014).

56

57 The ecological impacts of contaminated sites on macroinvertebrates have been documented in a few
58 studies where e.g. stream water samples were collected in the proximity of a contaminated
59 groundwater inflow zone and subsequently used for standard laboratory toxicity tests (Plotkin and
60 Ram 1984), or where benthic samples representing a dilution-mediated pollution gradient, i.e.
61 collected within the groundwater inflow zone and up to few hundred meters downstream, were
62 assessed (Dickman and Rygiel 1998). However, studies are still needed that investigate the
63 structural changes of especially sediment dwelling invertebrate communities along streams that are
64 intersected with contaminated groundwater inflow. Specifically, insight on the spatial extent of the
65 ecological effects of contaminated sites downstream of the primary contaminated groundwater
66 inflow zones is needed.

67
68 Benthic stream macroinvertebrates have traditionally been used as indicators for various
69 anthropogenic stressors since their sensitivity to these is high and their life span sufficiently long to
70 integrate effects (Rosenberg and Resh 1993). In European countries, the ecological quality
71 assessment is typically based on indices/metrics reflecting the effect of oxygen depletion due to the
72 degradation of non-toxic organic substances. One example is the ASPT (Average Score Per Taxon)
73 developed in the U.K. (Armitage et al. 1983) and modified for use in several other European
74 countries. The Danish Stream Fauna Index (DSFI) (Skriver et al. 2000) also belongs in this
75 category. Unfortunately, indices of this type generally have a low capability to capture the effects of
76 toxicants (Beketov and Liess 2008; Liess and von der Ohe 2005; McKnight et al. 2012). One
77 plausible explanation for this is that they are based on indicator species for high oxygen
78 concentrations, which is not necessarily linked to a high sensitivity for toxicants (Rubach et al.
79 2010). Consequently, alternative indices targeting e.g. organic toxicants (Beketov and Liess 2008)

80 and diffuse source pesticide pollution (Liess and von der Ohe 2005) have been developed to better
81 characterise the ecological effects of toxicants.

82

83 However, compound-specific standard toxicity data is not necessarily existing or available to an
84 extent that allows these types of ecological risk assessments of freshwater systems to be carried out.
85 Moreover, even when such data exists, current risk assessments based on laboratory and mesocosm
86 data tend to underestimate the ecological effects of e.g. pesticides observed in the field (Beketov et
87 al. 2013; Schäfer et al. 2012). There are indications that other chemicals, in spite of their predicted
88 low ecotoxicity such as chlorinated solvents, may also exacerbate the viability of stream
89 macroinvertebrate communities. For example, a recent study by Houde et al. (2015) revealed effects
90 of trichloroethylene (TCE) and vinyl chloride (VC) on genes and proteins related to metabolism,
91 reproduction and growth in *D. magna* at environmentally relevant concentrations (i.e. $\mu\text{g L}^{-1}$). In
92 consequence, using standard toxicity test data to predict field-effects of the continuous exposure of
93 chemicals with low predicted ecotoxicity will lead to inadequate quantification of the ecological
94 impacts that are only visible after successive generations of stream biota (*sensu* Liess and von der
95 Ohe (2005)).

96

97 The sediment dwelling macroinvertebrates, often dominated by species of Chironomidae (Diptera),
98 are potentially highly relevant stream indicators for contaminated groundwater inflow since they
99 reside in the contact zone between groundwater and surface water (Dickman and Rygiel 1998). The
100 taxon density of Chironomidae, however, does not necessarily reflect pollution gradients (Dickman
101 and Rygiel 1998; Lenat 1983), and species of especially Chironomidae differ widely in their habitat
102 preferences with respect to flow regimes, stream size and bed substrate composition (Lenat 1983;
103 Milosevic et al. 2013). Moreover, the duration of chironomid life-cycles varies strongly among

104 taxa, translating into a strong temporal variation in chironomid community structure. This
105 complicates the use of the taxonomic community composition of Chironomidae as a general
106 pollution indicator tool (Milosevic et al. 2013). Nevertheless, quantifying the community sensitivity
107 of all sediment dwelling macroinvertebrates to organic contaminants (*sensu* Beketov and Liess
108 (2008)) may provide a tighter link to the continuous exposure of contaminated groundwater inflow.

109

110 Morphological deformities of sediment dwelling Chironomidae larvae have additionally been
111 suggested as useful indicators for toxicant exposure to e.g. chlorinated compounds (Meregalli et al.
112 2001; Watts et al. 2001; Watts et al. 2003), pesticides (Gagliardi and Pettigrove 2013), metals (Di
113 Veroli et al. 2012; Dickman and Rygiel 1996), and as an effect screening tool for unknown
114 pollutants (Lenat 1993). The coupling between morphological deformities of Chironomidae and
115 ecologically relevant parameters (e.g. life cycle endpoints, reproductive output and population
116 dynamics) has not, however, been fully clarified. The use of *Chironomus* spp. as an indicator for
117 various pressures of chemical pollution may be partly constrained by naturally occurring high
118 background ratios of deformities, complicating the documentation of clear concentration-response
119 effects (Lenat 1993). Species of the genus *Prodiamesa* have lower background rates of
120 morphological deformities but may still respond to toxicant exposure (Servia et al. 1998). Thus,
121 *Prodiamesa* spp. may be a useful supplement to *Chironomus* spp. as a bioindicator for toxicant
122 stress.

123

124 We studied a 16 km reach of Grindsted stream (Denmark), where the stream flow is dominated by
125 groundwater inflow. Contaminated groundwater originating primarily from an old factory site in
126 Grindsted town discharges into the central part of the studied stream reach. Major contaminants
127 include chlorinated solvents and their metabolites, and pharmaceutical products. We sampled

macroinvertebrates at 7 sampling sites along the stream continuum representing an upstream control, contaminated groundwater discharge zones and a downstream dilution gradient. The overall aim of the study was to characterise macroinvertebrate communities at the sampling sites and evaluate the ability of currently used ecological indicator tools for characterising the contaminant effects. In addition, we aimed to characterise the spatial extent of the potential effect of a local groundwater-mediated contamination of the stream. We hypothesized that i) the contaminated groundwater would primarily affect macroinvertebrates in the contaminated groundwater discharge zones and not downstream sampling sites with elevated stream water concentrations and no contaminated groundwater inflow, ii) sediment dwelling macroinvertebrates respond more strongly to contaminated groundwater inflow compared to an analysis of the entire macroinvertebrate community, iii) the frequency of morphological deformities in *Chironomus* spp. and *Procladius* spp. increases in the contaminated groundwater discharge zone, and iv) the SPEARorganics index, targeting the effects of organic contaminants, provides a superior prediction of ecological effects of the investigated contaminants compared to currently used biotic indices (e.g. ASPT and DSFI).

142

143 MATERIAL AND METHODS

144 *Contaminated site at Grindsted and catchment characteristics*

145 The Grindsted factory site is located approximately 1.5 km from Grindsted stream (Fig. 1). The
146 Grindsted factory has produced various chemicals since 1914, including explosive material,
147 pharmaceutical compounds and enzymes (NIRAS 2009). More than 1,000 chemicals have been
148 handled at Grindsted factory during the last century, and large quantities of chemicals were
149 deposited within the factory site up to the mid-1990s. At present, the factory is not active and the
150 deposition of hazardous chemicals on the factory site has stopped. The contaminated site is
151 characterised by environmental toxicants including chlorinated solvents and their metabolites, as

152 well as pharmaceutical compounds such as barbiturates and sulphonamides (NIRAS, 2009), and
153 many of these contaminants are transported to Grindsted stream via groundwater (Nielsen et al.
154 2014).

155
156 The catchment of Grindsted stream is approximately 200 km² and is dominated by sand and sandy
157 clay (Heron et al. 1998). Agriculture and urban settings comprise approximately 54% and 12% of
158 the total catchment, respectively. The annual rainfall is 750-850 mm. The stream flow ranged from
159 1152 L/s⁻¹ to 2249 Ls⁻¹ from the up-gradient station S7 to the down-gradient station S1. The studied
160 stream reach receives only a small amount of surface water input via stream tributaries.

161
162 *Discharge zone identification, contaminant sampling and analyses*

163 The water temperature was systematically monitored along 50 m intervals at 50% and 100% of the
164 water depth, and 20 cm below the streambed (T₂₀) (Fig. S1a-b). The stream water temperature was
165 13-14 °C, and groundwater temperature 8-10 °C in August/September 2012. T₂₀ < 10 °C was thus
166 interpreted as a potential groundwater discharge zone, and confirmed by hydraulic head
167 measurements (Nielsen et al. 2014). Samples were extracted from the hyporheic zone (HZ) using
168 piezometers placed 40 cm below the streambed along the investigated stretch of Grindsted stream
169 (Fig. S1c). Moreover, stream water (SW) samples were manually collected in the middle of the
170 water column every 50-100 m (Fig. S2). HZ and SW samples were analysed for 9 chlorinated
171 solvents and 44 pharmaceutical compounds (barbiturates and sulfonamides; Table S1).

172
173 In total, 48 SW and 38 HZ samples were analysed for chlorinated solvents (Table 1). A subset of
174 these samples, based on the results from the chlorinated solvents, were then further analysed for
175 barbiturates and sulfonamides, specifically 5 SW and 2 HZ samples (Table S1). Samples for

176 chlorinated solvents were collected in 40 mL glass vials, preserved with 4M H₂SO₄ and stored at 4
177 °C. The analytes were extracted using the “head-space” method, and subsequently separated and
178 identified on a GC-MS (Agilent 7980 gas chromatograph equipped with an Agilent 5975 Celectron
179 impact (70eV) triple-axis mass-selective detector). Limits of detection and quantification were
180 determined following the methods provided in Winslow et al. (2006) (Table S1). We additionally
181 included SW data extracted from Petersen (2012) in order to improve the interpretive power when
182 linking toxicant concentrations to macroinvertebrate data (Table S2).

183

184 *Sampling sites*

185 We investigated seven sampling sites in this field study representing 15,800 m of Grindsted stream
186 (Fig. 1). The spatial allocation of sites was based on measured contaminant concentrations in stream
187 water sampled in autumn 2011 (Petersen 2012). We chose sampling sites near and downstream of
188 the discharge zone where contaminated groundwater (GW) from the Grindsted factory site was
189 found to enter SW. One additional site approximately 7,000 m upstream of the contaminated GW
190 discharge zone was included as a control (S7). One site was positioned just upstream of the
191 contaminated GW discharge zone (S6), two sites were positioned in the contaminated GW
192 discharge zone (S4 and S5), and the three final sites represented a dilution gradient along the stream
193 course downstream of the contaminated GW discharge zone (S1-S3) (Fig. 1). Each sampling site
194 refers to a 50 m reach used for macroinvertebrate sampling, physical characterisation and water
195 sampling for general water chemistry.

196

197 *Physical stream parameters*

198 At each sampling site the relative coverage of stones/boulders, gravel, sand and mud was estimated
199 along each of ten transects. These transects were evenly spaced by 5 m along the 50 m reach.

Moreover, we quantified the relative coverage of undercut banks (% of reach length), roots (% of reach length), high energy flow types (% of the 50 m reach), and emergent and submergent vegetation (% of the 50 m reach).

General water chemistry

Three 1 L water samples were collected manually at each of the seven sampling sites in September 2012 for analyses of general water chemistry. We measured biological oxygen demand (BOD₅), ammonia-N, nitrate-N, total-N (unfiltered samples), ortho-phosphate, total-P (unfiltered samples), suspended solids and the organic fraction of suspended solids. The BOD₅, ortho-phosphate and ammonia-N were analysed following their European Standards (DS/EN 1899 1999, DS/EN 1189-1997 and DS 11732 2005, respectively). Nitrate-N was analysed by applying the Lachat-method (Lachat Instruments, USA, Quickchem. No. 10-107-06-33-A (Salycate method)). Total-N and total-P were measured using the Kjeldahl-N method and Danish standard DS-291, respectively. Suspended solids were measured filtering water through a Whatman GFC filter (pore size 1.2 µm) and subsequently drying the filter at 105 °C for 24h. The organic fraction of the suspended solids was measured as the weight loss of the suspended solids after heating at 550 °C for 24h. Water temperature, conductivity and oxygen concentration were registered at each site using a multi-meter (WTW multi-350i) and pH was measured with a (YSI-60) pH-meter.

Macroinvertebrate sampling and identification

Macroinvertebrates were collected from all 7 sampling sites in September 2012 using a 500 cm² surber sampler attached to a shaft. At each sampling site, 12 surber samples were collected and pooled into one composite sample in the field, and were preserved using 96% ethanol. The surber samples were collected along three transects at 0, 25 and 50 m of the reach used for physical

224 characterisation. Along each transect, four surber samples were collected at 25, 50, 75 and 100% of
225 the distance from the left bank to the right bank.

226

227 All invertebrates were counted and identified to the level of species or genus (Table S2).

228 Chironomidae larvae were identified using the keys of Wiederholm (1983), Vallenduuk & Moller

229 Pillot (2007) and Moller Pillot (2009). Simuliidae larvae were identified using the key of Jensen

230 (1984), Trichoptera larvae were identified using the keys of Edington & Hildrew (1995) and

231 Wallace et al. (2003), and the remaining macroinvertebrates were identified using the keys provided

232 in Nilsson (2005).

233

234 *Mouthpart deformities of Chironomidae*

235 Mouthpart deformities in *Chironomus* spp. were not quantifiable because only four individuals

236 belonging to this genus were found, and they only represented the upstream control (S7) (Table S4).

237 We identified morphological deformities in *Prodiamesa olivacea* and focused on mentum and

238 antennae deformities (Al-Shami et al. 2010; Lenat 1993). Assessment of the severity of deformities

239 (slight, conspicuous, obvious) was performed according to Lenat (1993) and Servia et al. (1998).

240

241 *Data treatment*

242 Based on the macroinvertebrate data for each of the 7 sampling sites, we calculated the taxonomic

243 density, total abundance, Simpson index, Shannon index and Brillouin's index using PRIMER. In

244 addition, for each of these sampling sites, the Danish Stream Fauna Index (DSFI) score was

245 assessed following Skriver et al. (2000), the ASPT was assessed following Armitage et al. (1983)

246 and the SPEARorganics and SPEARpesticides index values were computed using the online and

247 freely available Species At Risk calculator (<http://www.systemecology.eu/spear/spear-calculator/>).

248 The SPEARorganics index is based on the sensitivities of macroinvertebrate taxa to synthetic
249 organic toxicants such as pesticides, surfactants and petrochemicals, and the index value represents
250 the general community tolerance to synthetic toxicants based on existing standardised toxicity tests
251 (Beketov and Liess 2008). Hence, we applied this measure to detect macroinvertebrate community
252 effects of the contaminants originating from the factory site. The SPEARpesticides index aims to
253 detect community changes due to periodic pesticide pollution typically occurring in agricultural
254 streams (Liess and von der Ohe 2005), and we applied this measure to evaluate the potential, but
255 not quantified, influence of diffuse source pesticide pollution from conventional agriculture.

256
257 The taxonomic density, total abundance, Simpson index, Shannon index and Brillouin's index were
258 computed for all sampled taxa, for sediment dwelling taxa and for Chironomidae, respectively.
259 Since ASPT and DSFI are not specifically developed for sediment dwelling taxa, we did not
260 compute these indices for this group of taxa. We calculated the SPEARpesticides values for all taxa,
261 since we used this index to evaluate general effects of diffuse source pesticide pollution. Lastly, we
262 did not calculate the SPEARorganics based on Chironomidae, since the data resolution behind the
263 SPEARorganics is currently insufficient to allow for a proper separation of the species of this
264 family (Beketov and Liess 2008).

265
266 The taxonomic macroinvertebrate community composition for each sampling site was furthermore
267 analysed using Nonmetric Multidimensional Scaling (NMDS) in PRIMER (Clarke and Warwick
268 2001). We scaled Bray-Curtis similarities based on 4th root transformations of species abundance
269 data to down weight dominant taxa, and ordinated using 100 runs. Analyses were performed
270 considering all taxonomic groups, as well as the isolated group of sediment dwelling organisms.

271

272 RESULTS AND DISCUSSION

273 *Contaminant concentrations*

274 The concentrations of chlorinated compounds within the region S3-S5 (Fig. 1) showed that
275 sampling site S4 was characterised by the highest contaminant concentrations in GW, confirming
276 that this area was an important discharge zone for the contaminated GW entering SW from the
277 factory site (Table 1). Specifically, the contaminant concentrations in the HZ at site S4 were a factor
278 of 2-50 higher than the concentrations found in the SW. In general, the concentrations of
279 chlorinated compounds in SW at site S5 were higher than those from the HZ at S5. Moreover, the
280 concentrations of chlorinated compounds in SW at site S5 were higher compared to those found in
281 SW at site S6. This indicates that contaminated GW additionally enters Grindsted stream between
282 sites S5 and S6. No contaminants associated with the contaminated factory site were detected in SW
283 at the upstream sites S6 and S7 in 2011 and 2012 (Tables 1, S2 and S3). Importantly, patterns
284 similar to those identified for the chlorinated solvents were found for concentrations of both the
285 sulfonamides and barbiturates (Tables 1, S2 and S3).

286

287 The concentrations of chlorinated compounds were found to continuously decrease in SW
288 downstream of site S4. Notably, VC was still detected at concentrations 4-20 times above the
289 Danish environmental quality standard (EQS) for surface water ($0.05 \mu\text{g L}^{-1}$, see Table S1) at site
290 S2 (5,000 m downstream of S4). The concentration of VC in the HZ at S4 exceeded the Danish
291 groundwater quality criterion ($0.2 \mu\text{g L}^{-1}$) by up to a factor of 1,000 (Table 1). The decreasing
292 concentrations of chlorinated compounds downstream of the site S4 probably reflect a combination
293 of volatilization (Aisopou et al. 2015) and dilution due to significant inflow of less contaminated
294 GW (see Table 3). The overall stream discharge increased approximately 100% from the upstream
295 location S7 to the downstream site S1.

296

297 Finally, it should be noted that the distribution of GW discharge zones and the contaminant
298 concentrations in the GW discharge into Grindsted stream may vary (Nielsen et al. 2012) and in
299 general, the spatial-temporal variation of groundwater can be relatively large (Anibas et al. 2011;).
300 Hence, especially the HZ samples may not always represent the highest hyporheic zone
301 concentrations in the sampling area.

302

303 *Physical properties and general water chemistry*

304 Physical parameters and general water chemistry are known to strongly influence macroinvertebrate
305 taxon density and community structure, but the sampling sites were highly comparable with respect
306 to the physical and chemical site properties (Tables 3 and 4). The substrate composition of the
307 sampling sites generally reflected the strong dominance of sandy soil types in the catchment with
308 sandy substrate dominating at all sites (Table 3). Whereas the relative coverage of sandy substrate
309 was > 70% for sites S1-S6, the upstream control site S7 was characterised by a higher substrate
310 complexity with considerable sediment fractions of gravel and mud, which may govern increased
311 macroinvertebrate taxonomic density (Kovalenko et al. 2012). Furthermore, submerged and
312 emergent vegetation covered 66% to 90% of the sampled reaches, which is typical for Danish low-
313 land streams (Baattrup-Pedersen and Riis 1999). Generally, the macrophyte community
314 composition is an important parameter governing macroinvertebrate taxon density (Ferreiro et al.
315 2014; Whatley et al. 2014). Although not quantified in this study, we observed that *Berula erecta*
316 and *Sparganium* sp. dominated the vegetation at all sites. Hence, we do not expect significant
317 differences in macrophyte community composition among sites. Finally, the general water
318 chemistry was also found to be highly comparable among sites (Table 4), and the measured
319 concentrations of macro-nutrients and BOD₅ are not expected to significantly influence the

320 macroinvertebrate community (Friberg et al. 2010). In summary, this indicates that contaminated
321 groundwater inflow, dominated by VC, cis-DCE and sulfonamides, were the most important factors
322 separating the sampling sites with respect to physical and chemical properties.

323

324 *Macroinvertebrate community responses*

325 The sites S4 and S5 were characterised by reduced taxa density, but only S4 was additionally
326 characterised by reduced diversity scores (Table 2) compared to both upstream as well as
327 downstream sites. Moreover, the EPT taxa density was reduced to four taxa at S4 compared to
328 upstream and downstream sites (5-10 taxa). These results probably reflect that site S4 contained the
329 highest concentrations of chlorinated and pharmaceutical compounds in both SW and the HZ (Table
330 1) suggesting that contaminated groundwater inflow is likely an important factor governing the
331 observed ecological impairment at site S4. In theory however, the taxonomic density should only be
332 compared if species accumulation curves for the samples have reached asymptotic equilibrium and
333 if a similar number of organisms have been collected (Gotelli and Colwell, 2001). Importantly, site
334 S4 was characterised by the highest total macroinvertebrate abundance (>5,000) compared to all
335 other sites (Table 2). This means that more organisms should have been collected at all other sites in
336 order to optimize the comparison of taxa density among sites, and this would likely have resulted in
337 an increase in taxa densities at these other sites. Hence, our results likely underestimate the impact
338 of the contaminated GW inflow on the total taxonomic density at site S4.

339

340 The NMDS analysis resulted in a strong two-dimensional ordination of the macroinvertebrate
341 communities (level of stress = 0.01) (Fig. 2). The sites S4 and S5, characterised by lower taxa
342 density and in part lower diversity (Table 2), were ordinated in close proximity to each other. There
343 was a tendency that sampling sites with increasing physical distance to S7 were ordinated with

344 increasing distance to S7 (Fig. 2). This could reflect that the contaminated GW discharging into
345 Grindsted stream around sites S4 and S5 may influence macroinvertebrate communities up to 8,000
346 meters downstream (site S1). However, various diffuse source urban pollutants (not covered in our
347 list of analytes), generally characterising urban settings (Roy and Bickerton 2012), may influence
348 macroinvertebrate communities downstream of Grindsted city and hence be important co-
349 explanatory factors for the observed deviation of downstream sites (Fig. 2).

350

351 *Sediment taxa*

352 The sediment dwelling taxa at sites S4 and S5 were characterised by lower taxonomical density and
353 diversity compared to upstream and downstream sampling sites (Table 2), thereby showing a
354 slightly more clear impairment compared to the macroinvertebrate community descriptors based on
355 all taxonomical groups. In contrast to the NMDS analysis of all taxonomical groups (Fig. 2), this
356 was additionally supported by the NMDS analysis of sediment dwelling taxa (level of stress < 0.01,
357 showing a very strong two-dimensional ordination) where sites S4 and S5 were clearly separated
358 from both up- and downstream sites (Fig. 3). These results suggest that the community composition
359 of sediment dwelling taxa may provide a stronger link to the contaminated GW inflow compared to
360 the full fauna samples. Intuitively, this is reasonable as sediment dwelling organisms reside deeper
361 in the groundwater-surface water interaction zone and are thus exposed to higher contaminant
362 concentrations than swimming and crawling taxa (Tables 1, S2 and S3).

363

364 The abundance of sediment dwelling organisms was approximately 10-fold higher at control site S7
365 compared to sites S1-S6 (Table 2), which is likely explained by a higher habitat complexity and in
366 particular, higher fractions of fine particulate organic material and mud (Table 3). Moreover, higher
367 fractions of mud at S7 could indicate higher sediment stability which, additionally, has a positive

368 influence. Sediment instability is an important constraining factor that should be carefully
369 considered in studies addressing the effects of GW contaminants on sediment dwelling
370 macroinvertebrates (Lenat et al. 1981).

371

372 In spite of the relatively clear differentiation of sediment dwelling macroinvertebrates at sites S4
373 and S5 (Fig. 3), generally few individuals of these taxa were found in all samples (Table 2), except
374 at site S7. The site S7 was characterised by higher fractions of fine particulate organic matter and
375 mud possibly indicating higher sediment stability, which has a positive influence on the abundance
376 of sediment dwelling organisms (Lenat et al. 1981). Moreover, inorganic sediments, dominating at
377 sites S1-S6, generally contain fewer taxa and lower abundances of sediment dwelling organisms
378 such as Chironomidae compared to sediments containing higher amounts of organic material
379 (Brunke and Gonser 1999). In consequence, the stringent focus on sediment dwelling
380 macroinvertebrates as bioindicators for contaminated GW in future monitoring and research efforts
381 could be confounded at sites with naturally high sediment instability and low content of particulate
382 organic carbon. We therefore suggest that future studies should additionally focus on meio-fauna
383 communities residing in the deeper and more stable parts of the sediments, as suggested by Brunke
384 and Gonser (1999). Importantly, meio-fauna community composition has been shown to change
385 along concentration gradients of other toxicants such as metals (Höss et al. 2011). Since inflowing
386 GW facilitates the clearing of finer sediment particles in groundwater-surface water interaction
387 zones, future studies should focus especially on sediment dwelling macroinvertebrates and meio-
388 fauna connected to habitats with low organic carbon content.

389

390 *Morphological deformities in Chironomidae larvae*

391 Mouthpart deformities were studied only in *Procladius olivacea*, since only four individuals of the
392 *Chironomus* spp. were detected in the fauna samples, and then only in the sample collected at S7. In
393 total, 24 specimens of *P. olivacea* were found of which only 3 had visible deformities of the
394 mentum ranked as classes I or II according to Servia et al. (1998). The morphological deformities
395 were associated with individuals from sites S5 and S6 (Table 2). Since the background deformity
396 rate of *P. olivacea* is low (~2%), these results insinuate effects of anthropogenic stress (Servia et al.
397 1998), but no contaminants from the factory site were detected at the site S6 precluding a clear
398 cause-effect relationship between the measured contaminant concentrations and mouthpart
399 deformity rates. However, since only two individuals of *P. olivacea* were found at sites S3-S5
400 (characterised by contaminated GW inflow), we cannot draw firm conclusions on the sensitivity of
401 morphological deformities of *P. olivacea* to the contaminated GW in this study. Other studies have
402 documented increased deformity rates in another species of Chironomida (*Chironomus riparius*)
403 exposed to organic contaminants (Meregalli et al. 2001; Watts et al. 2001; Watts et al. 2003).
404 Morphological deformities of Chironomidae have the potential to become a useful indicator of
405 ecological effects caused by GW contaminants, but obviously the use of such an effect indicator is
406 constrained to surface water bodies that naturally harbour higher abundances of the required
407 species. This important restriction could therefore disqualify the use and implementation of
408 morphological deformities of Chironomidae as general bioindicators for documenting the effects of
409 contaminated GW in monitoring programs.

410

411 *Macroinvertebrate indices*

412 All macroinvertebrate samples obtained a DSFI score of 5 indicative of “good ecological quality”
413 (Table 2). The number of positive diversity groups was highest at the upstream control site S7 and
414 lowest at site S4, but there was no clear link between the number of positive diversity groups and

415 measured contaminant concentrations. Thus the DSFI index, currently the only macroinvertebrate-
416 based index in use for assessing ecological quality in Denmark, did not reflect the observed
417 macroinvertebrate community changes at especially sites S4 and S5 (lower taxonomical density and
418 separation of the sites using multi-dimensional scaling (Figs. 2 and 3)). Similarly, the ASPT index
419 did not respond to the detected GW contaminants (Table 2). Similar findings were obtained by
420 McKnight et al. (2012) who studied the effects of a TCE and cis-DCE plume on the ecological
421 status of a stream. Consequently, alternative ecological indicator tools are needed for the
422 characterisation of ecological impacts of contaminated sites.

423
424 The SPEARorganics scores were comparable across sites S3-S6, and slightly higher compared to
425 sites S1-S2 and S7 (Table 2). Notably, higher scores are indicative of lower impact of organic
426 toxicants, but the scores for all seven sites remain within the expected range of reference sites (\geq -
427 0.4) as reported by Beketov and Liess (2008). The SPEARorganics index is based on LC₅₀ values
428 for freshwater macroinvertebrates exposed for 24h or 48h (data provided in von der Ohe and Liess
429 2004). Acute mortality data for *Daphnia magna* (Table S1) exists only for a few of the dominant
430 compounds detected in SW and the HZ in Grindsted stream (TCE, cis-DCE, VC and sulfonamides),
431 but they consistently have LC₅₀ concentrations $> 1 \text{ mg L}^{-1}$, thus being a factor 10 to 1,000 above the
432 measured concentrations in the HZ. The findings of Baun et al. (1999; 2000) further support the
433 prediction that the GW contaminants are not acutely lethal to *D. magna*. Comparing the
434 SPEARorganics results with the reduced taxonomic densities observed at sites S4 and S5 in
435 especially sediment dwelling species, this could imply that the predicted taxonomic sensitivities are
436 based on insufficient data to correctly identify sensitive populations. Equally important, the
437 substantial scarcity of available standard toxicity data most likely leads to incorrect effect/risk
438 predictions for the compounds detected in the GW plume at our study site. Considering the WFD-

439 related requirement for EU member states to evaluate the ecological impact of contaminated sites
440 on surface water bodies, the general lack of ecotoxicity data on the long-term effects of compounds
441 with low toxicity but long environmental persistence (partly facilitated through long-living sources),
442 poses an important problem that should receive increasing scientific and political attention.

443

444 The SPEARpesticides index was used to verify minimal expected impact of diffuse source pesticide
445 pollution originating from agricultural and urban sources, and the scores were generally high (28% -
446 43%, Table 2) indicating minimal impact by diffuse source pesticide pollution at the sampling sites
447 (Liess and von der Ohe 2005; von der Ohe et al. 2007).

448

449 *Conclusions*

450 Based on taxon density, diversity measures and NMDS analysis, we showed that macroinvertebrate
451 communities were impaired at the sampling sites S4 and S5 which were additionally characterised
452 by the highest contaminant concentrations in inflowing groundwater. Moreover, we showed that the
453 changes in macroinvertebrate communities were strongest for the fraction of sediment dwelling
454 taxa. Interestingly, none of the currently used macroinvertebrate indices applied in this study,
455 including SPEARorganics, could identify this ecological impairment. This could be due in part to a
456 suboptimal classification of taxonomic sensitivities to organic pollutants in the SPEARorganics
457 index, since e.g. the contaminants characterising the groundwater plume at Grindsted factory site
458 have low toxicity and generally have been tested on a very limited number of supplemental test
459 organisms. Morphological deformities in *P. olivacea* only occurred at the sites S5 and S6, but the
460 total number of individuals was too low to draw firm conclusions on the mutagenic effects of the
461 groundwater plume.

462

463 We conclude that the environmental setting of contaminants with low toxicity to invertebrates
464 continuously discharging into streams from long-lived sources is not sufficiently reflected in the
465 current standard ecotoxicity testing program. A reliable estimate for the physiological sensitivity of
466 organisms to toxicants is essential for any ecological indicator of toxicant effects. However, the
467 production of such toxicity data can become pragmatically, as well as financially challenging.
468 Future studies on ecological effects of contaminated sites could address this problem through i)
469 identifying response patterns in ecological and morphological traits of macroinvertebrates that may
470 be more sensitive than taxonomical endpoints to contaminant pollution (Doledec and Statzner 2008;
471 Statzner and Bêche 2010), and ii) exploring responses in the meio-fauna communities residing in
472 the hyporheic zone where exposure concentrations are higher, and subsequently linking these
473 responses to the benthic macroinvertebrate communities.

474

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483

484 REFERENCES

485 Aisopou A, Bjerg PL, Sonne AT, Balbarini N, Rosenberg L, Binning PJ (2015) Dilution and
 486 volatilization of groundwater contaminant discharges in streams. J Contam Hydrol 172: 71-
 487 83.

488 Al-Shami S, Rawi CSM, Nor SAM, Ahmad AH, Ali A (2010) Morphological deformities in
 489 *Chironomus* spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of
 490 anthropogenic and environmental stresses on three rivers in the Juru river system, Penang,
 491 Malaysia. Env Entomol 39:210-222.

492 Anibas C, Buis K, Verhoeven R, Meire P, Batelaan O (2011) A simple thermal mapping method for
 493 seasonal spatial patterns of groundwater-surface water interaction. J Hydrol 397:93-104.

494 Armitage PD, Moss D, Wright JF, Furse MT (1983) The performance of a new biological water
 495 quality score system based on macroinvertebrates over a wide range of unpolluted running
 496 water sites. Water Res 17:333-347.

497 Baun A, Jensen SD, Bjerg PL, Christensen TH, Nyholm N (2000) Toxicity of organic chemical
 498 pollution in groundwater downgradient of a landfill (Grindsted, Denmark). Env Sci Technol
 499 34:1647-1652.

500 Baun A, Kloft L, Bjerg PL, Nyholm N (1999) Toxicity testing of organic chemicals in groundwater
 501 polluted with landfill leachate. Env Toxicol Chem 18:2046-2053.

502 Beketov MA, Liess M (2008) An indicator for effects of organic toxicants on lotic invertebrate
 503 communities: Independence of confounding environmental factors over an extensive river
 504 continuum. Env Pol 156:980-987.

505 Beketov MA, Kefford BJ, Schäfer RB, Liess M (2013) Pesticides reduce regional biodiversity of
 506 stream invertebrates. Proc Nat Ac Sci USA 110: 11039-11043.

507 Brunke M, Gonser T (1999) Hyporheic invertebrates-the clinal nature of interstitial communities
 508 structured by hydrological exchange and environmental gradients. *J N Am Ben Soc* 18:344-
 509 362.

510 Baattrup-Pedersen A, Riis T (1999) Macrophyte diversity and composition in relation to substratum
 511 characteristics in regulated and unregulated Danish streams. *Freshwat Biol* 42:375-385.

512 Chapman SW, Parker BL, Cherry JA, Aravena R, Hunkeler D (2007) Groundwater-surface water
 513 interaction and its role on TCE groundwater plume attenuation. *J Contam Hydrol* 91:203-
 514 232.

515 Clarke KR, Warwick RM (2001) Change in marine communities: An approach to statistical analysis
 516 and interpretation, 2nd edn. PRIMER-E Ltd., Plymouth Marine Laboratory, UK

517 Conant B, Cherry JA, Gillham RW (2004) A PCE groundwater plume discharging to a river:
 518 influence of the streambed and near-river zone on contaminant distributions. *J Contam*
 519 *Hydrol* 73:249-279.

520 Daughton CG (2005) "Emerging" chemicals as pollutants in the environment: a 21st century
 521 perspective. *Renew Res J* 23:6-23.

522 Di Veroli A, Goretti E, Paumen ML, Kraak MHS, Admiraal W (2012) Induction of mouthpart
 523 deformities in chironomid larvae exposed to contaminated sediments. *Env Pol* 166:212-217.

524 Dickman M, Rygiel G (1996) Chironomid larval deformity frequencies, mortality, and diversity in
 525 heavy-metal contaminated sediments of a Canadian Riverine Wetland. *Env Int* 22:693-703.

526 Dickman M, Rygiel G (1998) Municipal landfill impacts on a natural stream located in an urban
 527 wetland in regional Niagara, Ontario. *Can F-Nat* 112:619-630.

528 Doledec S, Statzner B (2008) Invertebrate traits for the biomonitoring of large European rivers: an
 529 assessment of specific types of human impact. *Freshwat Biol* 53:617-634.

530 Edington JM, Hildrew AG (1995) Caseless caddis larvae of the British Isles with notes on their
 531 ecology. Freshwater Biological Association, The Ferry House, Far Sawrey, Ambleside,
 532 Cumbria LA22 0LP

533 Ferreira N, Feijoo C, Giorgi A, Rosso J (2014) Macroinvertebrates select complex macrophytes
 534 independently of their body size and fish predation risk in a Pampean stream. *Hydrobiol*
 535 740:191-205.

536 Friberg N, Skriver J, Larsen SE, Pedersen ML, Buffagni A (2010) Stream macroinvertebrate
 537 occurrence along gradients in organic pollution and eutrophication. *Freshwat Biol* 55:1405-
 538 1419.

539 Gagliardi B, Pettigrove V (2013) Removal of intensive agriculture from the landscape improves
 540 aquatic ecosystem health. *Agric Ecosyst Env* 176:1-8.

541 Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: Procedures and pitfalls in the measurement
 542 and comparison of species richness. *Ecol Lett* 4: 379-391.

543 Heron G, Bjerg PL, Gravesen P, Ludvigsen L, Christensen TH (1998) Geology and sediment
 544 geochemistry of a landfill leachate contaminated aquifer (Grindsted, Denmark). *J Contam*
 545 *Hydrol* 29:301-317.

546 Höss S, Claus E, Von der Ohe PC, et al. (2011) Nematode species at risk - A metric to assess
 547 pollution in soft sediments of freshwaters. *Env Int* 37:940-949.

548 Houde M, Douville M, Gagnon P, Sproull J, Cloutier F (2015) Exposure of *Daphnia magna* to
 549 trichloroethylene (TCE) and vinyl chloride (VC): Evaluation of gene transcription, cellular
 550 activity, and life-history parameters. *Ecotoxicol Env Saf* 116: 10-18.

551 Jensen F (1984) A revision of the taxonomy and distribution of the Danish black-flies (Diptera:
 552 Simuliidae), with keys to the larval and pupal stages. *Natura Jutlandica* 21:69-116.

553 Kovalenko KE, Thomaz SM, Warfe DM (2012) Habitat complexity: approaches and future
554 directions. *Hydrobiol* 685:1-17.

555 Lenat DR (1983) Chironomid taxa richness: Natural variation and use in pollution assessment.
556 *Freshwat Inv Biol* 2:192-198.

557 Lenat DR (1993) Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity
558 and organic loading in streams. *J N Am Bent Soc* 12:265-269.

559 Lenat DR, Penrose DL, Eagleson KW (1981) Variable effects of sediment addition on stream
560 benthos. *Hydrobiol* 79:187-194.

561 Liess M, von der Ohe PC (2005) Analyzing effects of pesticides on invertebrate communities in
562 streams. *Env Toxicol Chem* 24:954-965.

563 McKnight US, Funder SG, Rasmussen JJ, Finkel M, Binning PJ, Bjerg PL (2010) An integrated
564 model for assessing the risk of TCE groundwater contamination to human receptors and
565 surface water ecosystems. *Ecol Eng* 36:1126-1137.

566 McKnight US, Rasmussen JJ, Kronvang B, Binning PJ, Bjerg PL (2015) Sources, occurrence and
567 predicted aquatic impact of legacy and contemporary pesticides in streams. *Env Pol* 200:64-
568 76.

569 McKnight US, Rasmussen JJ, Kronvang B, Bjerg PL, Binning PJ (2012) Integrated assessment of
570 the impact of chemical stressors on surface water ecosystems. *Sci Tot Env* 427-428:319-
571 331.

572 Meregalli G, Pluymers L, Ollevier F (2001) Induction of mouthpart deformities in *Chironomus*
573 riparius larvae exposed to 4-n-nonylphenol. *Env Pol* 111:241-246.

574 Milosevic D, Simic V, Stojkovic M, et al. (2013) Spatio-temporal pattern of the Chironomidae
575 community: toward the use of non-biting midges in bioassessment programs. *Aquat Ecol*
576 47:37-55.

577 Milosevic N, Thomsen NI, Juhler RK, Albrechtsen H-J, Bjerg PL (2012) Identification of
 578 discharge zones and quantification of contaminant mass discharges into a local stream from
 579 a landfill in a heterogeneous geologic setting. *J Hydrol* 446:13-23.

580 Moller Pillot HKM (2009) Chironomidae larvae: Biology and ecology of the Chironomini. KNNV
 581 Publishing, Koninklijke Wöhrmann BV, The Netherlands.

582 Nielsen SS, Tuxen N, Frimodt O, et al. (2014) Risk assessment of surface water impacted by point
 583 source related contaminated groundwater (In Danish). Danish EPA, Copenhagen, Denmark,
 584 176 pp.

585 Nilsson A (2005) Aquatic insects of Northern Europe: A taxonomic handbook. Apollo Books.

586 NIRAS (2009) A historical overview of the contamination in Grindsted (In Danish). Technical
 587 Report No 04/350. Ribe County.

588 Petersen MF (2012) Quantification and risk assessment of continuous micropollutant mass
 589 discharges from multiple sources to a gaining stream at the catchment scale. Master Thesis,
 590 Technical University of Denmark.

591 Plotkin S, Ram NM (1984) Multiple bioassays to assess the toxicity of a sanitary landfill site. *Arch*
 592 *Env Cont Toxicol* 13:197-206.

593 Rasmussen JJ, Baattrup-Pedersen A, Larsen SE, Kronvang B (2011) Local physical habitat quality
 594 cloud the effect of predicted pesticide runoff from agricultural land in Danish streams. *J Env*
 595 *Monit* 13:943-950.

596 Rasmussen JJ, McKnight US, Loinaz MC, et al. (2013) A catchment scale evaluation of multiple
 597 stressor effects in headwater streams. *Sci Tot Env* 442:420-431.

598 Rosenberg DM, Resh VH (1993) Freshwater biomonitoring and benthic macroinvertebrates
 599 Chapman & Hall, London.

600 Roy JW, Bickerton G (2012) Toxic Groundwater Contaminants: An Overlooked Contributor to
 601 Urban Stream Syndrome? *Env Sci Technol* 46:729-736.

602 Rubach MN, Baird DJ, Van den Brink PJ (2010) A new method for ranking mode-specific
 603 sensitivity of freshwater arthropods to insecticides and its relationship to biological traits.
 604 *Env Toxicol Chem* 29:476-487.

605 Schäfer RB, von der Ohe PC, Rasmussen JJ, Kefford B, Beketov M, Schulz R, Liess M (2012)
 606 Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in
 607 streams. *Env Sci Technol* 46: 5134-5142.

608 Servia MJ, Cobo F, Gonzalez MA (1998) Deformities in larval *Prodiamesa olivacea* (Meigen,
 609 1818) (Diptera, Chironomidae) and their use as bioindicators of toxic sediment stress.
 610 *Hydrobiol* 385:153-162.

611 Skriver J, Friberg N, Kirkegaard J (2000) Biological assessment of running waters in Denmark:
 612 introduction of the Danish Stream Fauna Index (DSFI). *Verh Int V Limnol* 27:1822–1830.

613 Statzner B, Bêche LA (2010) Can biological traits resolve effects of multiple stressors on running
 614 water ecosystems? *Freshwat Biol* 55:80-119.

615 Tesoriero AJ, Duff JH, Saad DA, Spahr NE, Wolock DM (2013) Vulnerability of Streams to
 616 Legacy Nitrate Sources. *Env Sci Technol* 47(8):3623-3629

617 Vallenduuk HJ, Moller Pillot HKM (2007) Chironomidae larvae: General ecology and
 618 Tanypodinae. KNNV Publishing, De Budelse bv, Budel, The Netherlands.

619 von der Ohe PC, Liess M (2004) Relative sensitivity distribution of aquatic invertebrates to organic
 620 and metal compounds. *Env Toxicol Chem* 23:150-156

621 von der Ohe PC, Prub A, Schafer RB, Liess M, de Deckere E, Brack W (2007) Water quality
 622 indices across Europe - a comparison of the good ecological status of five river basins. *J Env*
 623 *Monit* 9:970-978.

624 Wallace ID, Wallace B, Phillipson GN (2003) Keys to the case-bearing caddis larvae of Britain and
625 Ireland. Freshwater Biological Association.

626 Watts MM, Pascoe D, Carroll K (2001) Chronic exposure to 17 alpha-ethinylestradiol and
627 bisphenol A-effects on development and reproduction in the freshwater invertebrate
628 *Chironomus riparius* (Diptera : Chironomidae). *Aquat Toxicol* 55:113-124.

629 Watts MM, Pascoe D, Carroll K (2003) Exposure to 17 alpha-ethinylestradiol and bisphenol A-
630 effects on larval moulting and mouthpart structure of *Chironomus riparius*. *Ecotoxicol Env*
631 *Saf* 54:207-215.

632 Weatherill J, Krause S, Voyce K, Drijfhout F, Levy A, Cassidy N (2014) Nested monitoring
633 approaches to delineate groundwater trichloroethene discharge to a UK lowland stream at
634 multiple spatial scales. *J Contam Hydrol* 158:38-54.

635 Westbrook SJ, Rayner JL, Davis GB, Clement TP, Bjerg PL, Fisher ST (2005) Interaction between
636 shallow groundwater, saline surface water and contaminant discharge at a seasonally and
637 tidally forced estuarine boundary. *J Hydrol* 302:255-269.

638 Whatley MH, van Loon EE, van Dam H, Vonk JA, van der Geest HG, Admiraal W (2014)
639 Macrophyte loss drives decadal change in benthic invertebrates in peatland drainage ditches.
640 *Freshwat Biol* 59:114-126.

641 Wiederholm T (1983) Chironomidae of the Holarctic region, vol 19. *Entomologica Scandinavica*,
642 Borgströms Tryckeri AB, Motala, Sweden.

643 Winslow SD, Martin JJ, Hallberg GR, et al. (2006) Statistical procedures for the determination and
644 verification of minimum reporting levels for drinking water methods. *Env Sci Technol*
645 40:281-288.

646

647 Table 1. Concentrations of selected chemicals in stream water (SW) and water extracted from the hyporheic zone (HZ). Where appropriate,
648 ranges of values are given, representing water samples collected at the seven sampling sites in Grindsted stream (± 50 m) in 2011 (Table
649 S2) and 2012 (Table S3). NA stands for no available data.

Macroinvertebrate sampling site (distance from S7 (m))	S1 (15,844)	S2 (13,344)	S3 (10,894)		S4 (7,952)		S5 (7,594)		S6 (7,171)	S7
	SW	SW (n=2)	SW (n=2)	HZ	SW (n=2)	HZ (n=2)	SW (n=2)	HZ	SW	SW
<i>Chlorinated solvents</i>										
PCE ($\mu\text{g L}^{-1}$)	<0.04	0.07-0.09	0.08-0.13	0.35	0.13-0.18	0.1*	0.08	0.05	<0.04	<0.04
TCE ($\mu\text{g L}^{-1}$)	<0.04	0.050-0.06	0.05-0.09	0.26	0.11-0.16	0.15-0.40	0.05-0.06	0.04	<0.04	<0.04
cis-DCE ($\mu\text{g L}^{-1}$)	0.14	1.20-1.41	1.50-2.07	2.54	2.89-5.01	4.40-9.30	0.29-0.33	0.23	<0.05	<0.05
Vinyl chloride ($\mu\text{g L}^{-1}$)	0.11	0.44-1.09	0.54-2.13	0.23	3.22-4.59	43-252	0.34-0.36	0.21	<0.05	<0.05
<i>Pharmaceutical compounds</i>										
Sulphanilamide ($\mu\text{g L}^{-1}$)	NA	1.1	1.5**	NA	NA	24**	NA	NA	<1.0	<1.0
Sulfaguanidine ($\mu\text{g L}^{-1}$)	NA	0.58-0.80	0.84**	NA	NA	18**	NA	NA	<0.5	<0.5
Sulfamethazine ($\mu\text{g L}^{-1}$)	NA	0.97-1.1	1.3**	NA	NA	<0.5**	NA	NA	<0.5	<0.5
Sulfamethiazole ($\mu\text{g L}^{-1}$)	NA	0.60-1.20	1.4**	NA	NA	<0.05**	NA	NA	<0.05	<0.05
Sulfadiazin ($\mu\text{g L}^{-1}$)	NA	0.03-0.08	0.025**	NA	NA	<0.05**	NA	NA	<0.05	<0.05
Sulfanilic acid ($\mu\text{g L}^{-1}$)	NA	1.90*	2.4**	NA	NA	1.0**	NA	NA	<0.5	<0.5
Barbital ($\mu\text{g L}^{-1}$)	NA	<1.0	1.2	NA	NA	6.8**	NA	NA	NA	NA
Isopropylbarbituric acid ($\mu\text{g L}^{-1}$)	NA	<1.0	<1.0	NA	NA	9.5**	NA	NA	NA	NA

650 * Concentration of the compound > LOQ for only one of the replicate samples

651 ** Concentrations of the pharmaceutical compounds are based on one sample

652 Table 2. Macroinvertebrate metrics and diversity measures for the seven sampling sites

	S1	S2	S3	S4	S5	S6	S7
<i>All taxonomic groups</i>							
Taxonomic density	25	27	23	13	17	25	31
Abundance (ind. m ⁻²)	605	1022	1577	5473	2175	3745	4220
Simpson index	0.75	0.71	0.75	0.50	0.75	0.62	0.66
Shannon index	1.79	1.73	1.77	1.05	1.67	1.18	1.79
Brillouin index	1.70	1.66	1.73	1.04	1.20	1.37	1.57
# EPT taxa	7	6	8	4	8	11	11
EPT abundance	16	32	205	133	108	528	193
ASPT	5.27	5.13	5.46	6.00	6.08	5.86	5.31
DSFI score	5	5	5	5	5	5	5
Pos. div. groups (DSFI)	7	5	6	4	8	8	10
Neg. div. groups (DSFI)	1	1	2	0	0	1	4
SPEARorganics	-0.37	-0.34	-0.19	-0.19	-0.23	-0.18	-0.35
SPEARpesticides	40.23	34.94	43.09	42.17	41.92	45.47	28.84
<i>Sediment taxa</i>							
Taxonomic density	8	7	6	3	2	5	6
Abundance (ind. m ⁻²)	32	40	35	12	8	22	227
Simpson index	0.73	0.67	0.70	0.61	0.32	0.75	0.60
Shannon index	1.69	1.43	1.46	1.00	0.50	1.46	1.12
Brillouin's index	1.29	1.21	1.17	0.71	0.32	1.11	1.06
SPEARorganics	-0.55	-0.41	-0.45	-0.37	-0.36	-0.38	-0.39
<i>Chironomidae</i>							
Taxonomic density	6	7	6	4	1	5	8
Abundance (ind. m ⁻²)	28	40	35	20	2	23	72
Simpson index	0.67	0.71	0.70	0.58	0	0.73	0.66
Shannon index	1.43	1.51	1.46	1.08	0	1.43	1.36
Brillouin's index	1.10	1.22	1.17	0.81	0	1.10	1.16
<i>P. olivacea</i> deformities	0/9	0/5	0/0	0/1	1/1	2/4	0/1
(#deformities/total # ind)							

653

654

655

656 Table 3. Physical characteristics of the seven sampling sites (S1-S7) in Grindsted stream. Each
657 value is given \pm Std. dev. (n = 10). Discharge was measured at stations S1, S5 and S7 (n = 4).

Parameter	S1	S2	S3	S4	S5	S6	S7
Wetted width (m)	7.9 \pm 0.8	8.9 \pm 0.9	10.2 \pm 1.0	10.3 \pm 1.5	10.1 \pm 1.2	10.3 \pm 1.5	15.5 \pm 2.4
% boulder	0	0	0	3 \pm 5	0	0	1 \pm 2
% gravel	16 \pm 11	11 \pm 15	0	13 \pm 9	11 \pm 9	5 \pm 5	21 \pm 16
% sand	82 \pm 10	81 \pm 16	94 \pm 5	83 \pm 12	85 \pm 7	88 \pm 8	34 \pm 12
% mud	2 \pm 5	8 \pm 10	6 \pm 6	1 \pm 3	4 \pm 3	7 \pm 9	44 \pm 17
% roots	0	20 \pm 9	0	0	1 \pm 1	0	0
% submerged vegetation	29 \pm 10	50 \pm 11	46 \pm 14	72 \pm 23	72 \pm 20	65 \pm 19	36 \pm 9
% emerged vegetation	27 \pm 5	21 \pm 11	27 \pm 11	13 \pm 5	12 \pm 5	20 \pm 10	54 \pm 10
% undercut banks	0	39 \pm 12	0	3 \pm 5	3 \pm 5	3 \pm 5	5 \pm 11
% high energy flow	9 \pm 9	10 \pm 6	8 \pm 5	14 \pm 14	13 \pm 14	14 \pm 14	7 \pm 5
Discharge (L s ⁻¹)	2249 \pm 16	NA	NA	NA	1892 \pm 19	NA	1152 \pm 2

658

659 Table 4. Chemical and physicochemical properties of the seven sampling sites (S1-S7) in Grindsted
660 stream.

Parameter	S1	S2	S3	S4	S5	S6	S7
<i>Water chemistry</i>							
Ammonia-N (mg L ⁻¹)	0.043	0.076	0.099	0.098	0.096	0.099	0.114
Nitrate-N (mg L ⁻¹)	3.14	2.95	2.91	3.28	3.26	3.28	3.08
Total N (mg L ⁻¹)	3.16	2.99	3.1	3.4	3.38	3.55	3.21
ortho-phosphate (mg L ⁻¹)	0.018	0.03	0.011	0.013	0.013	0.012	0.012
Total P (mg L ⁻¹)	0.102	0.116	0.078	0.075	0.073	0.08	0.079
BOD ₅ (mg O ₂ L ⁻¹)	1.28	1.37	1.44	1.44	1.34	1.4	1.9
Susp. particles (mg L ⁻¹)	2.7	2.9	2.4	2.7	3.6	2.5	1.9
Susp. organic particles (mg L ⁻¹)	1.3	1.5	1.3	1.5	1.6	1.3	1.1
<i>Physicochemical properties</i>							
pH	6.52	6.68	6.52	6.56	6.5	6.52	6.75
Temperature (°C)	13.4	13.5	13.4	13.1	13.2	12.9	12.8
Conductivity (μS cm ⁻¹)	274	286	264	272	273	272	237

661

662 Figure captions:

663

664 Fig. 1. Schematic overview of the seven sampling sites in Grindsted stream and the contaminated
665 factory site.

666

667 Fig. 2. NMDS plot including all taxonomic groups detected in the fauna samples for the seven
668 sampling sites. The level of stress was 0.01.

669

670 Fig. 3. NMDS plot including sediment dwelling taxa detected in the fauna samples for the seven
671 sampling sites. The level of stress was < 0.01 .

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